Volatile Organic Compounds as Breath Biomarkers for Active and Passive Smoking

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We used real-time breath measurement technology to investigate the suitability of some volatile organic compounds (VOCs) as breath biomarkers for active and passive smoking and to measure actual exposures and resulting breath concentrations for persons exposed to tobacco smoke. Experiments were conducted with five smoker/nonsmoker pairs. The target VOCs included benzene, 1,3-butadiene, and the cigarette smoke biomarker 2,5-dimethylfuran. This study includes what we believe to be the first measurements of 1,3-butadiene in smokers' and nonsmokers' breath. The 1,3-butadiene and 2,5-dimethylfuran peak levels in the smokers' breath were similar (360 and 376 μg/m^3, respectively); the average benzene peak level was 522 μg/m^3. We found higher peak values of the target chemicals and shorter residence times in the body than previously reported, probably because of the improved time resolution made possible by the continuous breath measurement method. The real-time breath analyzer also showed the presence of the chemicals after exposure in the breath of the nonsmokers, but at greatly reduced levels. Single breath samples collected in evacuated canisters and analyzed independently with gas chromatography/mass spectrometry confirmed the presence of the target compounds in the postexposure breath of the nonsmokers but indicated that there was some contamination of the breath analyzer measurements. This was likely caused by desorption of organics from condensed tar in the analyzer tubing and on the quartz fiber filter used to remove particles. We used the decay data from the smokers to estimate residence times for the target chemicals. A two-compartment exponential model generally gave a better fit to the experimental decay data from the smokers than a single-compartment model. Residence times for benzene, 1,3-butadiene, and 2,5-dimethylfuran ranged from 0.5 (1,3-butadiene) to 0.9 min (benzene) for τ_1 and were essentially constant (14 min) for τ_2. These findings will be useful in models of environmental tobacco smoke exposure and risk.
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Tobacco smoke causes lung cancer and has been classified as a group A carcinogen under the U.S. Environmental Protection Agency's (EPA) carcinogen assessment guidelines (*1*). Various studies have shown that tobacco smoke is an important source of several well-established carcinogens, including benzene, 1,3-butadiene, and *N*-nitrosamines (*2–11*).

Exposure to tobacco smoke-related chemicals is widely believed to pose significant health risks. Studies suggest, for example, that prenatal or childhood passive exposure to parents' smoking significantly increases the risk of childhood and adult cancers (*12–15*), and nonsmoking spouses of smokers have an increased risk of lung and nasal sinus cancers compared to spouses of nonsmokers (*1,16,17*). According to Wallace (*18,19*) and Krause et al. (*20*), indoor benzene concentrations are, respectively, about 50% and 69% higher in smokers' homes than in the homes of nonsmokers. Furthermore, tobacco smoke is responsible for 5% of the total exposure to benzene in the United States. Smokers receive 89% of their benzene exposure directly from smoking; nonsmokers derive about 10% of their exposure from environmental tobacco smoke (ETS) (*21*). Benzene is a hematotoxic substance that can cause acute myeloid leukemia and has been classified as a group A human carcinogen by EPA (*22*).

Cigarette smoke and engine exhausts are the major sources of exposure of the general population, through inhalation, to 1,3-butadiene. EPA has identified 1,3-butadiene as a group B2 probable human carcinogen (*23*) based on evidence of carcinogenicity from studies in humans, which indicate a causal relationship between occupational exposure to 1,3-butadiene and excess mortality from lymphatic and/or hematopoietic cancers (*23,24*). There is concern over 1,3-butadiene because it has a unit risk factor 30 times greater than that for benzene (*22,23*).

Previous studies have shown that active cigarette smoking directly affects the levels of benzene and other volatile organic compounds (VOCs) in breath and blood (*25–28*). Because of the dynamic equilibrium between the concentration of a VOC in the blood and its concentration in exhaled breath (*29*), breath measurements can be used to estimate body burden and to detect changes in body burden with time (*30–33*). Although benzene in exhaled air is quite a good indicator of active smoking (*34*), there are too many other sources of benzene in the environment to make it an effective marker for the gas phase of ETS or an indicator of passive smoking (*35*). Attempts to use nicotine or cotinine as biomarkers of dose are sometimes criticized on the grounds that nicotine changes phase from gas to solid as cigarette smoke ages, and it deposits on surfaces at variable rates that depend on local conditions (*36,37*). It has therefore been argued that nicotine may not be a good marker for either the gas or particle phase of ETS.

In 1990, Gordon (*38*) evaluated the gas-phase constituents of smokers' breath in an attempt to identify components that could serve as definitive markers of smoking. Of the 200-plus chemicals detected in the breath samples of 26 smokers and 43 nonsmokers, 2,5-dimethylfuran was found to be a strong indicator of smoking status. Recently, Ashley et al. (*39*) analyzed blood samples from smokers and nonsmokers and showed that 2,5-dimethylfuran is equally effective as a blood biomarker for smoking.

Little is known about the actual levels of tobacco smoke-related chemicals in smokers' breath. Information on nonsmokers is even more sparse. In this pilot study, therefore, we sought to fill this data gap by using continuous real-time breath measurement technology (*30,40*) to investigate breath concentrations in active and passive smokers of the cigarette smoke-associated VOCs benzene and 1,3-butadiene and the smoker breath biomarker VOC 2,5-dimethylfuran (*38*). Our approach was to isolate a smoker and nonsmoker in a small, unventilated

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room and have the smoker smoke four cigarettes in his/her normal manner, with a 20- to 25-min interval between ending one cigarette and beginning another. After each puff, the smoker first emptied his or her lungs of smoke, then breathed into the real-time breath analyzer while the uptake and decay of the target chemicals in the blood was monitored. After each of the first three cigarettes, we recorded the longer-term decay of the chemicals in the smoker's breath. On completion of the fourth and final cigarette, we monitored the nonsmoker's breath for the same chemicals and determined the increases in levels of chemicals caused by the ETS in the room.

Methods

Exposure conditions and breath sampling protocol. The experiments, which were conducted with five adult smoker/nonsmoker pairs, were designed to measure the uptake and decay in real time of the cigarette-associated target VOCs in the exhaled breath of each smoker and determine whether exposure to the resulting ETS was measurable in the breath of the nonsmoker.

The smoker subjects were requested to refrain from smoking on the morning of the experiment. For each experiment, a smoker and nonsmoker were isolated in a small (24.9 m^3), unventilated room. Before exposure to the first cigarette began, the smoker and nonsmoker each provided baseline breath samples by breathing unfiltered room air and exhaling via a standard disposable mouthpiece into the breath inlet of the real-time breath analyzer (*30,40*). We used these samples to determine background breath concentrations for each target analyte.

Exposure was initiated by having the smoker light his or her first cigarette and puff normally on the cigarette. After each puff, the first breath exhalation was vented to the room air to clear the lungs of smoke and avoid drawing particulate matter into the breath analyzer. Then the subject directed the second and subsequent exhalations into the breath analyzer so that the short-term, postpuff uptake and decay of the target chemicals in the smoker's blood could be monitored. These measurements recorded the maximum breath concentrations of the compounds as a function of the individual puffs taken. Each subject smoked four cigarettes, five to seven puffs per cigarette, during a 2- to 2.5-hr *ad libitum* smoking period. After the final puff, the smoker immediately put on a full face mask (Model 8932; Hans Rudolph, Inc., Kansas City, MO), and the longer-term decay of the chemicals in the smoker's breath was recorded continuously for approximately 15 min, while the subject inhaled hospital-grade breathing air supplied to the mask from a gas cylinder. After a break of about 5–10 min, the smoker repeated the entire sequence two more times. On completion of the fourth and final cigarette, we monitored the nonsmoker's breath for the same chemicals with the real-time breath analyzer and, immediately thereafter, collected a breath sample using the single breath canister method (*41*). Canister samples were subsequently analyzed by automated gas chromatography/mass spectrometry (GC/MS) using a modified version of U.S. EPA Method TO-14 (*42*).

We recruited subjects in pairs through advertisements in the local university newspaper. To be eligible, each volunteer pair had to consist of an active smoker and a nonsmoker companion. Because this was a small convenience sample, it was not meant to be representative of any particular population, and we made no attempt to demographically match the smoker/nonsmoker pairs.

Information on the participants is provided in Table 1, along with a summary of the experimental conditions. The study protocol was reviewed and approved by the Battelle Human Subjects Committee, and informed consent was obtained from each subject before their participation. Subjects were free to smoke their usual brand of cigarette. We felt that forcing them to smoke a single standardized brand with which they were unfamiliar, in an effort to avoid variability in smoke composition, would likely have altered their smoking behavior. Smokers tend to be very loyal to their brand choices and attach significant imagery to particular cigarette brands. That imagery may well affect the way they smoke a cigarette if a brand is not their usual cigarette.

Before the first cigarette was lit and after cigarettes were smoked during the exposure sessions, we collected whole-air samples in the middle of the room using evacuated 6-L stainless-steel canisters and analyzed the samples by GC/MS (*42*). The mean background concentration for benzene in the room was 2.2 ± 0.5 (SD) $\mu g/m^3$; background levels for 1,3-butadiene and 2,5-dimethylfuran were below the detection limits, which for these whole-air samples were 0.6 $\mu g/m^3$ for benzene, 0.4 $\mu g/m^3$ for 1,3-butadiene, and 2.4 $\mu g/m^3$ for 2,5-dimethylfuran. At the start of the experiments, the air exchange rate for the room was determined using the standard sulfur hexafluoride tracer technique (*43*).

The subjects were requested to refrain from smoking and avoid coffee in the morning before participating in the experiment. [2,5-dimethylfuran has been identified by headspace analysis as a volatile component of roasted Jamaican coffee beans (*44*), although its presence in the exhaled breath of coffee drinkers has not been established.]

Breath analysis. We have developed special monitoring technology to obtain better time resolution over the uptake and elimination periods of an exposure episode (*30,40*). The technology can measure trace VOCs in exhaled breath or air continuously in real time. It consists of a breath inlet unit, a quartz fiber filter (0.25-µm pore size), a direct breath sampling interface (glow discharge ionization source), and a compact ion trap mass spectrometer (ITMS) that is capable of operation in the full tandem (MS/MS) mass spectrometric mode (*40,45–48*). This direct breath sampling/mass spectrometric approach offers a powerful means of extracting and quantifying VOCs directly from the breath matrix, and eliminates the preconcentration step that normally precedes exhaled air analysis by conventional GC/MS (*31,49*). To define the uptake of a compound of interest, all that is required is to use the real-time breath analyzer to measure and record the breath levels of a subject continuously from the start of the exposure. Information on decay rates may similarly be obtained from data generated during the elimination phase.

The quartz filter, precleaned in a muffle furnace, was added to the system to prevent the influx of cigarette smoke particles, which would otherwise block the inlet orifice of the glow discharge source. Before the start of each exposure session with a subject pair, we replaced the quartz filter with a clean filter,

Table 1. Information on subjects for cigarette smoke exposure study.[a]

Smoker/nonsmoker pair no.	Smoker/nonsmoker sex	Cigarette brand[b]	Smoker/nonsmoker age (years)	Smoker/nonsmoker height (m)	Smoker/nonsmoker weight (kg)	Smoker's ventilation rate (L/min)[c]
1	M/M	A	20/20	1.75/1.73	68.0/68.0	12.0 ± 2.0
2	M/M	B	20/22	1.79/1.83	90.7/72.6	11.2 ± 2.5
3	M/M	C[d]	26/38	1.65/1.63	59.0/119.3	12.4 ± 2.1
4	F/F	A	20/25	1.57/1.68	56.7/52.2	4.1 ± 0.6
5	F/F	D	20/20	1.65/1.57	59.0/56.7	7.1 ± 0.5

[a]Volume of room in which experiments were conducted, 24.9 m^3; air exchange rate, 0.62 hr^{-1}; average room temperature, 25.7 ± 0.9°C. [b]All cigarette brands filtered, low tar. [c]Average. [d]Mentholated cigarette; all other nonmentholated cigarettes.

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and the face mask and tubing to the breath sampling interface were washed with soap and water, rinsed with methanol, and air-dried for at least 12 hr.

For all of the breath measurements undertaken in this study, except those immediately following the smoker's final puff from a cigarette, a disposable mouthpiece containing a one-way valve was attached to the breath inlet to provide a constant source of exhaled air for the mass spectrometer (*30,40*). When using the mouthpiece, the subject inhaled room air through the nose and exhaled into the mouthpiece through the mouth.

After the smoker's final puff, a Rudolph Model 8932 face mask, which contains a two-way non-rebreathing valve set to isolate the smoker from the ETS in the room, was substituted for the mouthpiece. The exhalation valve from the face mask was connected directly to the breath inlet system by means of a 15.2-cm (6 in) length of 22-mm (0.9 in) diameter corrugated polyethylene tubing (Allied Healthcare, Inc., Toledo, OH). The inlet valve of the face mask was connected to a 15-L polyethylene bag that served as a buffer volume and was attached to a cylinder containing hospital-grade breathing air. We used a special head cap joined to the face mask to ensure a secure and comfortable fit of the mask to the subject's face and head and to reduce strain on the neck muscles. The entire flow system from the mouthpiece to the mass spectrometer was heated to prevent condensation of exhaled water vapor and minimize surface losses of trace-level organics in the exhaled air.

The undiluted breath sample is vacuum-extracted at a constant rate from the breath inlet by the vacuum in the breath sampling interface and flows into the ion trap without any attention from the subject. The volume of the breath inlet is approximately 95 mL, or roughly one-fifth the mean value of the adult tidal volume. Thus, each breath exhalation effectively displaces the previous breath sample, and a steady gas flow is maintained into the analyzer. This ensures that unit resolution is achieved between individual breath exhalations while, at the same time, producing a constant and undiluted sample for analysis. We used a dry gas meter (Model DTM-115; American Meter Co., Horsham, PA), attached to the vent of the breath inlet system via wide-bore flexible tubing, to record the total exhaled volume of breath from each subject.

As noted earlier, the direct breath sampling interface is a glow discharge ionization source, which is attached to the ITMS. The operation of this system has been described in detail elsewhere (*47,48,50*). The analyzer is a Teledyne Electronic Technologies (Mountain View, CA) 3DQ Discovery ion trap MS, a compact, field-deployable instrument with high sensitivity and specificity. We measured all the target analytes selected for this investigation in the MS/MS mode. The parent and product ions that were selected and monitored were *m/z* 78 and 51 for benzene, *m/z* 54 and 39 for 1,3-butadiene, and *m/z* 95 and 67 for 2,5-dimethylfuran. The ion trap was operated in the dual scan mode, in which two scan functions are used alternately to control the instrument. In this mode, the data acquired for *m/z* 51 (benzene) and 39 (1,3-butadiene) from scan function 1 were stored as the Odd scans, and the data acquired for *m/z* 67 (2,5-dimethylfuran) from scan function 2 were stored as the Even scans. The ion trap was set to record the average mass spectra for the target ions once every 6 sec in each scan mode. Because the levels of the target compounds were relatively high in the smokers' breath, we did not adjust the ion trap for maximum sensitivity (*51,52*). Consequently, sensitivity was not optimal for the measurement of these compounds in the breath of the nonsmokers.

The breath analyzer was calibrated with gas standards of the target compounds that were prepared in a 186-L glass chamber. In addition, we collected 6-L samples from the chamber in evacuated stainless-steel canisters and analyzed the samples independently for confirmation using a standard method (*42*). We calibrated the instrument each day before the experiment got underway, then after each cigarette was smoked.

Background levels for the target analytes, which were estimated by passing humidified ultrahigh purity air through the entire breath analyzer and measuring the signals at the masses used to monitor the compounds, were below the detection limits for all three compounds. Limits of detection, estimated by taking three times the standard deviation of the background concentration, were 6.9 μg/m^3 for benzene, 3.0 μg/m^3 for 1,3-butadiene, and 7.5 μg/m^3 for 2,5-dimethylfuran. We set the limit of quantitation for each compound at three times the detection limit (*53*).

To determine the precision of the real-time technique, we used the glass chamber as a constant source and recorded the variation in MS/MS ion signal. By averaging the results over a suitable time interval, we estimated mean values and standard deviations for the target analytes, thus providing an overall measure of system stability and reproducibility. Over a 3.5-hr period, this yielded a relative standard deviation of 2.2%. Accuracy was determined by spiking known volumes of 2,5-dimethylfuran and benzene into humidified zero-grade air in the 186-L glass chamber, then withdrawing 6-L sample aliquots into evacuated stainless-steel canisters which were analyzed independently, using GC/MS and traceable standards where available. The measured concentration as a fraction of the expected value was 82% for benzene and 93% for 2,5-dimethylfuran. We quantified all concentrations measured using the real-time system with the results obtained from the GC/MS analysis of the ITMS gas calibration standards.

Data analysis. We used the linear mass-balance multicompartment model, developed by Wallace et al. (*32*), to assess the decay of the target compounds in the blood and estimate residence times. The compartment model was also used to evaluate important parameters that define the uptake of the compounds of interest.

For exposure to a constant high concentration, C_{air}, the multicompartment model has the following solution:

$$C_{br} = fC_{air}\sum a_i\left(1 - e^{\frac{-t}{\tau_i}}\right), \qquad [1]$$

where C_{br} = exhaled breath concentration of the component; C_{air} = concentration of the component in inhaled air; f = fraction of inhaled breath concentration exhaled at equilibrium; a_i = fractional contribution of the ith compartment to the breath at equilibrium ($\Sigma a_i = 1$); t = time from the onset of exposure; and τ_i = residence time of the chemical in the ith compartment. (C_{br} is the mixed expired air, consisting both of alveolar air and the portion of the pure air supply that did not undergo alveolar exchange.)

During the postexposure decay phase, the breath concentration declines exponentially:

$$C_{br} = \sum a_i e^{\frac{-t}{\tau_i}}, \qquad [2]$$

where, now, t is measured from the time exposure ends. In Equation 2, the first term (compartment) is generally associated with blood, the second with "highly perfused tissues," the third with "moderately perfused tissues," and the fourth with "poorly perfused tissues." For a broad range of VOCs, it has been found that the residence times for these compartments are roughly similar, namely, 3–11 min for the first compartment, 0.4–1.6 hr for the second, 3–8 hr for the third, and several days for the fourth compartment (*54*). The residence time is defined as the time it takes for the compound to decay to $1/e$ of its initial concentration in the compartment, assuming all other compartments are at zero concentration. The biological half-life, $t_{1/2}$, of the compound in the body is related to the residence time, τ, through the expression $\tau = t_{1/2}/\ln 2$. For the relatively short exposure times involved in smoking cigarettes, only the first and second

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compartments are likely to make measurable contributions to the breath levels during the decay phase.

We determined all the parameters by first fitting the breath decay data using the Marquardt-Levenberg (nonlinear regression) algorithm (SigmaPlot Version 5.0; SPSS, Chicago, IL), which minimizes the differences in the sum of squares between the assumed model and the experimental data. This provided values for the a_i and τ_i terms defined in Equation 2.

Wallace et al. (*54*) have provided a solution to the multicompartment model for the case of an exposure at relatively high concentrations for a time T followed immediately by exposure to clean air:

$$C_{br} = fC_{air}\sum a_i\left(1 - e^{\frac{-t}{\tau_i}}\right) \quad (t \le T) \qquad [3]$$

$$C_{br} = fC_{air}\sum a_i\left(1 - e^{\frac{-t}{\tau_i}}\right) e^{\frac{-(t-T)}{\tau_i}} \quad (t \ge T), \qquad [4]$$

where t = time from the start of the exposure, and T = total duration of an exposure to a constant concentration C_{air}.

Cigarette smoking does not normally subject the smoker to a constant exposure concentration. Instead, exposure experienced by a smoker is cyclic—smoking a single cigarette followed by an exposure-free period before the next cigarette is lit. It is therefore reasonable to assume that these discrete activities produce a succession of rises and declines in the breath levels during the exposure and postexposure periods. As a result, the breath levels at the end of each exposure or postexposure segment are described by the following set of equations (*55,56*):

$$C_{br}(t_1) = fC_{air}\sum a_i\left[1 - e^{\frac{-(t_1-t_0)}{\tau_i}}\right] \qquad [5]$$

$$C_{br}(t_2) = fC_{air}\sum a_i\left[1 - e^{\frac{-(t_1-t_0)}{\tau_i}}\right] e^{\frac{-(t_2-t_1)}{\tau_i}} = C_{br}(t_1)e^{\frac{-(t_2-t_1)}{\tau_i}} \qquad [6]$$

$$C_{br}(t_3) = fC_{air}\sum a_i\left[1 - e^{\frac{-(t_2-t_2)}{\tau_i}}\right] + C_{br}(t_2)e^{\frac{-(t_3-t_2)}{\tau_i}} \qquad [7]$$

$$C_{br}(t_4) = C_{br}(t_3)e^{\frac{-(t_4-t_3)}{\tau_i}} \qquad [8]$$

Similarly, for n exposure/postexposure periods, the equations are:

$$C_{br}(t_{n-1}) = fC_{air}\sum a_i\left[1 - e^{\frac{-(t_{n-1}-t_{n-2})}{\tau_i}}\right] + C_{br}(t_{n-2})\,e^{\frac{-(t_{n-1}-t_{n-2})}{\tau_i}} \qquad [9]$$

$$C_{br}(t_n) = C_{br}(t_{n-1})e^{\frac{-(t_n-t_{n-1})}{\tau_i}} \qquad [10]$$

In these equations, t_0 denotes the beginning of the first exposure (puff) period, t_1 denotes the end of the first exposure period, and $(t_1 - t_0)$ is the duration of the first exposure period. Similarly, $(t_2 - t_1)$ denotes the duration of the first postexposure (or breath sampling) period. In the same manner, $(t_3 - t_2)$ denotes the second exposure period, and $(t_4 - t_3)$ is the second postexposure period. In general, $(t_{n-1} - t_{n-2})$ denotes the $(n-2)$th exposure period, and $(t_n - t_{n-1})$ represents the $(n-2)$th postexposure period. Equations 5–10 are used to simulate the concentrations in the smokers' breath as a result of the cyclic exposure experienced while smoking successive cigarettes separated by brief exposure-free intervals.

Results

Smokers. We used the breath analyzer to monitor the levels of the target analytes in the exhaled breath of five active smokers after each individual puff and during the decay period after they finished each cigarette. Figure 1 shows a typical breath concentration/time profile for benzene, 1,3-butadiene, and 2,5-dimethylfuran from one of the volunteer smokers. Immediately after each puff, the smoker first exhaled the smoke, then breathed into the analyzer, and measurements were taken for 1–2 min to record the maximum breath levels of the compounds. Each puff sequence in the plot represents an episode in which the smoker first drew on the cigarette and exhaled the smoke (valley portion of peak). Then, while inhaling room air, the smoker breathed normally into the analyzer until the signal passed through a maximum and began to decrease into the next valley, when the next puff was taken. After the final puff from each of the first three cigarettes, the subject put on the face mask, and we monitored the elimination of the compounds from the breath continuously for about 15 min, until the signal had returned roughly to its original baseline level.

The plots in Figure 1 and those obtained for the other smokers monitored show that the measured breath signals for the target chemicals closely track each other, suggesting that the breath benzene and 1,3-butadiene levels were highly correlated with the breath levels of the smoke biomarker 2,5-dimethylfuran. The occurrence of benzene in the exhaled breath of both smokers and nonsmokers is well established (*25–28,34,35*). However, to the best of our knowledge, these are the first reported measurements of

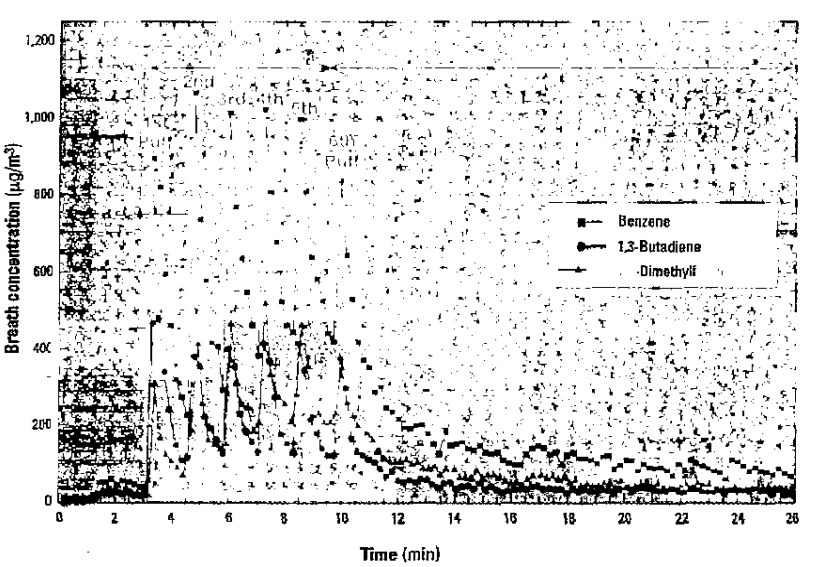

Figure 1. Continuous exhaled breath concentration/time profiles for benzene, 1,3-butadiene, and 2,5-dimethylfuran, obtained from smoker 4 (female, age 20 years) while smoking the third cigarette (filtered, low tar).

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1,3-butadiene in human exhaled breath. Two striking features of these plots are the high maximum breath concentrations measured for the target compounds after each puff and the very rapid decreases in concentration that occurred for each compound in the period immediately after active exposure to the mainstream smoke ended.

Figure 2 shows the average maximum post-puff breath concentrations of the target chemicals for each cigarette as a function of the number of cigarettes smoked by the smoker from Figure 1 (smoker 4) during the 2- to 2.5-hr exposure session. The maximum concentration of each compound increased rapidly with the first cigarette smoked, and then stayed roughly constant with the remaining cigarettes smoked in the exposure event. Figure 3 presents the maximum breath concentrations of the analytes of interest in the breath of the active smokers, averaged over the four cigarettes smoked by each smoker. The average maximum breath concentration for benzene varied widely with smoker, from a low value of 128 μg/m^3 (for smoker 1) to a high value of 886 μg/m^3 (for smoker 4). In contrast, the average breath level for 1,3-butadiene was relatively constant with smoker, averaging 373 ± 51 (SD) μg/m^3; for 2,5-dimethylfuran, the average breath level was 375 ± 150 μg/m^3.

The breath decay curves after the final puff from each of the first three cigarettes smoked by each active smoker were fitted to the one- and two-compartment curves described by Equation 2. As an example, the experimentally measured decay curves are compared with the modeled curves in Figure 4 for smoker 5 after smoking the third cigarette. The curves show that the breath concentrations for all of the chemicals of interest decreased extremely rapidly with time immediately after exposure.

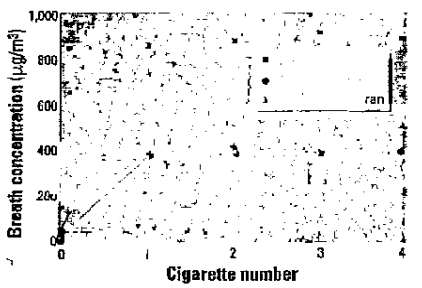

Figure 2. Average maximum postpuff breath concentration of target chemicals as a function of the number of cigarettes smoked during the exposure session by smoker 4. Breath concentrations corresponding to cigarette number 0 were measured immediately before the first cigarette was smoked.

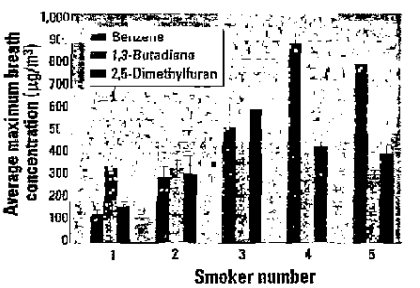

Figure 3. Average maximum postpuff breath concentration for benzene, 1,3-butadiene, and 2,5-dimethylfuran in exhaled breath of active smokers, averaged over four cigarettes smoked during each exposure session. Error bars indicate SD.

The mean residence times, τ_i, as calculated from the experimental data, are presented for the target chemicals in Table 2. Values obtained for τ_1 for benzene, 1,3-butadiene, and 2,5-dimethylfuran range from 0.47 min (1,3-butadiene) to 0.88 min (benzene); those for τ_2 are essentially constant at 14 min. Estimates of the mean values for the coefficients, a_i, of the exponential terms are also given in Table 2. Fractional mean values for a_1 range from 0.69 (2,5-dimethylfuran) to 0.88 (1,3-butadiene). For a_2, mean values range from 0.12 (1,3-butadiene) to 0.31 (2,5-dimethylfuran).

ETS and biomarkers in nonsmokers' breath. **Room air measurements.** Measurements of the air exchange rate for the room, made using the standard tracer technique, yielded a value of 0.62 ± 0.07 (SD) air changes per hour. During the exposure sessions with subject pairs 1–3, room air samples were taken before the first cigarette was smoked and immediately after each cigarette was finished by each smoker to determine the air concentrations of the compounds of interest. In the case of subject pairs 4 and 5, only two samples were collected each time during the experiments: the first before the first cigarette was smoked, and the second after either the third (subject pair 4) or fourth cigarette (subject pair 5).

We collected grab samples each time in the middle of the room using 6-L stainless-steel canisters. The measured buildup in the room air levels of the target compounds during each exposure session is presented in Figure 5. For 1,3-butadiene and 2,5-dimethylfuran, the air concentrations in all cases were undetectable before the first cigarette is smoked; those for benzene were typical of indoor concentrations (*21*). For all three compounds, the levels increased with the number of cigarettes smoked. The plots in Figure 5 suggest that the concentrations leveled off after the third or fourth cigarette. The average room air concentrations after the third and fourth cigarettes are 22.1 ± 6.5 (SD) μg/m^3 for benzene and 18.9 ± 9.9 μg/m^3 for 1,3-butadiene; the 2,5-dimethylfuran levels in the room air are much lower, on average 5.0 ± 1.3 μg/m^3.

The room air concentrations for benzene and 1,3-butadiene measured in the present study are similar to those reported in some earlier studies (*2,9,19,20,34*), although actual concentrations are strongly dependent on room volume, number of cigarettes smoked, air exchange rates, and so on. In our study,

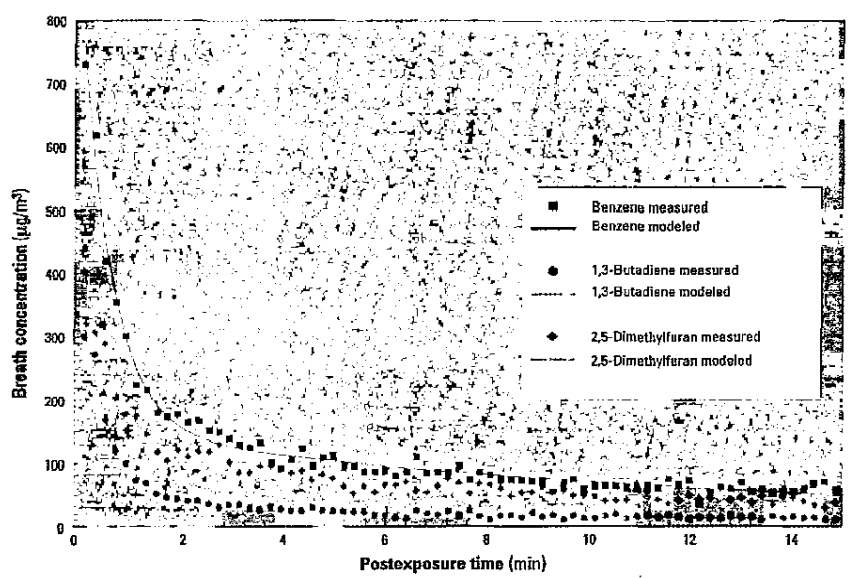

Figure 4. Measured and modeled decay of target chemicals in exhaled breath for smoker 5 after smoking third cigarette.

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we used a small room and adjusted the ventilation rate to a level that is typical of residential levels (*57*), in an attempt to obtain quantifiable ETS-related exposure levels in the breath of the nonsmokers in the room. Under these conditions, the average concentration of benzene due to ETS in our experimental room was 19.9 μg/m^3 (22.1–2.2 μg/m^3 background), about five times the average concentration of benzene due to ETS (3.5–4.5 μg/m^3) previously measured in a large number of smokers' homes (*19,20*).

Exhaled breath measurements. To establish whether exposure to ETS is measurable in the breath of the nonsmokers, we determined the pre- and postexposure breath levels of the target analytes using the real-time breath analyzer. Before the first cigarette was lit in the room, a preexposure breath sample was taken from the smoker and the nonsmoker. After the smoker's fourth cigarette, a postexposure breath sample again was obtained from the nonsmoker. At that time, we also collected a whole-breath sample for GC/MS analysis by bypassing the breath analyzer and using an evacuated stainless-steel canister. The breath analyzer and canister results obtained for the nonsmokers from subject pairs 1, 2, 4, and 5 are presented in Figure 6. Data are not included here for nonsmoker 3 because of a contamination problem that occurred with the breath samples taken from this subject.

Discussion

Smokers. **Breath concentration/time profiles.** The maximum concentrations of the target analytes in the breath increase rapidly with the first cigarette smoked, then stay roughly constant with the remaining cigarettes smoked during the exposure event. Figure 3 summarizes the maximum concentrations of the chemicals in the breath of the five participating smokers, averaged over the four cigarettes smoked by each smoker.

The effects of cigarette smoking on breath levels of benzene have been examined in previous studies. In the Total Exposure Assessment Methodology (TEAM) study, the concentration of benzene in breath increased significantly with the number of cigarettes smoked (on the day of the breath measurement) (*34*). A maximum benzene concentration of 47 μg/m^3 was obtained for smokers who smoked more than 50 cigarettes per day. The average benzene breath concentration of smokers in the TEAM study was, however, only 14 μg/m^3 (*18*). Several other studies have been reported, including an on-line study, in which the levels of benzene in smokers' breath were measured (*27,58–62*). Breath benzene concentrations in these studies ranged from about 5 to 90 μg/m^3. In most of these investigations, breath measurements commenced at some time after smoking ceased. In the on-line work reported by Jordan et al. (*59*), proton transfer-reaction mass spectrometry (PTR-MS) was used to rapidly measure benzene and acetonitrile in the breath of smokers. Immediately before smoking a cigarette, the breath benzene levels were essentially the same as those for nonsmokers. Right after smoking one cigarette, the benzene concentration rose to about 82 μg/m^3 and then decreased rapidly to the presmoking level.

These values are significantly lower than the breath benzene levels observed in the present study. As noted earlier, plots such as those in Figures 1 and 4 show that the breath benzene concentration is strongly time dependent, falling extremely rapidly with time immediately after each puff is taken. As a result, the amount of benzene that will be measurable in the breath is a strong function of the time elapsed since the last draw on the cigarette. In the present study, we started breath measurements just seconds after each puff was exhaled, resulting in a more precise definition of the peak maximum and much higher measured concentrations of benzene than have been reported in any previous investigation.

Our measured average maximum concentrations for benzene and 1,3-butadiene in the breath of the participating smokers may be compared with expected values based on the inhalable amount present in mainstream smoke. Two of the cigarette brands used by the subjects in the current study were included in the recently completed 1999 Massachusetts Benchmark Study (*63*). The

Table 2. Average model decay parameters (mean ± SD) for target chemicals in breath of five smokers, plus adjusted R^2 (range).

Compound	a_1 (μg/m^3)	a_2 (μg/m^3)	τ_1 (min)	τ_2 (min)	Adjusted R^2
Benzene	444 ± 201	160 ± 61	0.88 ± 0.51	14 ± 4	0.929–0.992
1,3-Butadiene	287 ± 92	40 ± 14	0.47 ± 0.09	14 ± 4	0.953–0.995
2,5-Dimethylfuran	281 ± 159	129 ± 67	0.78 ± 0.22	14 ± 6	0.943–0.987

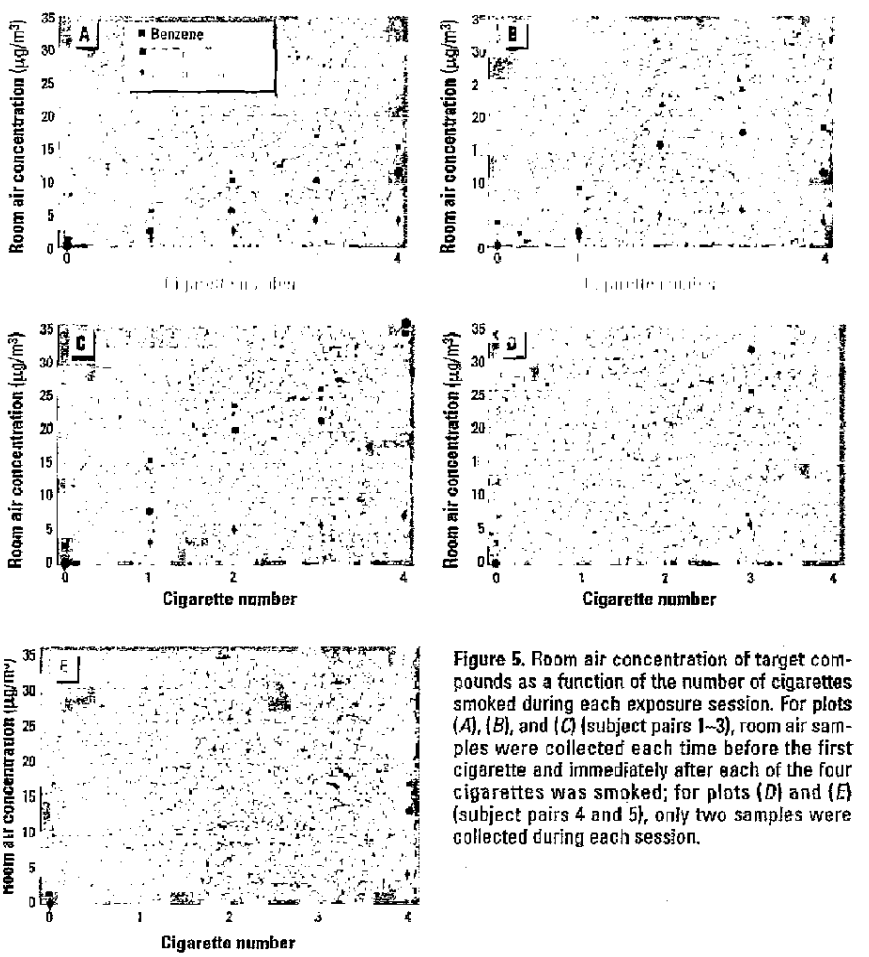

Figure 5. Room air concentration of target compounds as a function of the number of cigarettes smoked during each exposure session. For plots (*A*), (*B*), and (*C*) (subject pairs 1–3), room air samples were collected each time before the first cigarette and immediately after each of the four cigarettes was smoked; for plots (*D*) and (*E*) (subject pairs 4 and 5), only two samples were collected during each session.

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Benchmark Study, which was undertaken to characterize smoke constituent yield data for cigarette brands commonly sold in the United States, involved 26 commercial cigarette brand styles: 6 mentholated and 20 nonmentholated. Two of the 26 styles tested were nonfiltered cigarettes. Standard smoke-yield parameters (i.e., tar, nicotine, and carbon monoxide) were measured along with mainstream and sidestream yields for 41 selected smoke constituents, including benzene and 1,3-butadiene. If we assume that the 24 filtered cigarette styles tested are representative of the cigarettes smoked by the subjects in this study, then the average Benchmark Study tar content of the cigarettes smoked here was 10.6 mg/cigarette, which corresponds to mainstream smoke yields of 76 and 74 µg/cigarette for benzene and 1,3-butadiene, respectively. These values are slightly higher than the yields of 56 µg per cigarette (range 42–73 µg) for benzene and 65 µg per cigarette (range 54–75 µg) for 1,3-butadiene obtained earlier by Brunnemann et al. (*2,3*).

If it takes a typical smoker 10 puffs to smoke a cigarette, and the volume of each puff is equal to twice the normal tidal volume (~500 mL) (*57*), then the inhaled concentration from a cigarette is estimated to be 7,600 µg/m^3 for benzene and 7,400 µg/m^3 for 1,3-butadiene. Although the estimated inhaled concentrations for benzene and 1,3-butadiene are similar, the unit risk factor for 1,3-butadiene is about 30 times higher than that for benzene (*22,23*), so that the risk associated with exposure to 1,3-butadiene as a result of active smoking is about 30 times that for benzene. If we further assume now that the fractions of inhaled breath benzene and 1,3-butadiene that are exhaled unchanged at equilibrium are, respectively, equal to about 36% (*32,62*) and 10% (*64*), the estimated exhaled levels are 2,736 µg/m^3 for benzene and 740 µg/m^3 for 1,3-butadiene. Although our measured average maximum breath values for these chemicals are significantly higher than any previously recorded in the breath of smokers, they are still substantially lower than these estimated levels. It is important to note, however, that under typical smoking conditions, the concentrations in the blood and exhaled breath are nearly always far from equilibrium, so that the fraction exhaled will be much smaller than predicted by the value of f. If, for example, the benzene concentration in the blood is low, then nearly all of the inhaled benzene will go into the blood. We therefore expect our observed values to be a good deal less than predicted under equilibrium conditions.

Decay curves and residence times. Our measurements show that the breath concentrations of the target compounds decrease extremely rapidly with time immediately after each puff. After the final puff from each of the first three cigarettes smoked by each smoker, continuous breath measurements of the decay phase were taken from the smoker for about 15 min while the concentrations of the compounds of interest decreased to concentrations that approached the preexposure levels. The recent PTR-MS on-line measurements of Jordan et al. (*59*) also showed that the benzene concentration is extremely time dependent, falling rapidly with time after the last puff on a cigarette. In Jordon et al.'s study, however, breath measurements were made 4–5 minutes apart, and the rate of decrease was not as rapid as in our study; their reported breath levels returned to the preexposure levels in about 1 hr.

Mean residence times, calculated from the experimental data, yielded values for τ_1 that range from 0.5 min (for 1,3-butadiene) to 0.9 min (for benzene), while those for τ_2 are essentially constant at 14 min. Similar short residence times for benzene in compartment 1 were predicted by Wallace et al. (*32*) (2–4 min) and have since been determined in breath by Lindstrom and Pleil (*65*) (2–4 min), Gordon et al. (*30*) (4–6 min), and Yu and Weisel (*62*) (9 min). Sato et al. (*66*), using a three-compartment model for benzene elimination from the blood, obtained a value of 2 min for τ_1. Independent determinations with which to compare our residence time for 1,3-butadiene in compartment 1 have not been reported in the literature. However, Wallace et al. (*4,29*) have pointed out that τ_1 is on the order of 3–12 min for a number of common nonpolar VOCs representing three chemical classes (aromatic, aliphatic, and chlorinated compounds). We can similarly compare our residence time of 0.8 min for the polar VOC, 2,5-dimethylfuran, in compartment 1 with values reported in the literature for the polar VOC, methyl *tert*-butyl ether, that range from 2 to 4 min (*55,65*).

Wallace et al. (*4,29*) also showed that typical residence times for compartment 2 are on the order of 18–120 min for several aromatics, aliphatics, and chlorinated VOCs. Our measurements yielded a value for τ_2 of 14 min for benzene, 1,3-butadiene, and 2,5-dimethylfuran. Specific values of τ_2 for benzene in compartment 2 that have been reported by others include 43 and 581 min (*65*), 176 min (*30*), and 98 min (*62*); Sato et al. (*66*) obtained a value for τ_2 of 30 min for benzene elimination from the blood. The shorter residence times obtained in the present study are likely due to the smaller time resolution and larger number of measurements made, which allowed us to extract these times more accurately. The relatively small number of discrete samples taken in previous studies limited the accuracy and probably caused some combination of two residence times into one of intermediate length, whereas the long sample-turnaround times that occurred in the earliest chamber studies prevented any measurement of the short first-residence times.

Modeling inhalation exposure from smoking. We used the estimates for the residence times and the coefficients of the exponential terms for benzene in Table 2, along with Equations 9 and 10, to construct a model of the benzene concentrations in smokers' breath as a result of the short-term cyclic exposure that occurs when smoking a single cigarette. We set the fraction f in Equation 9 equal to 0.36 (*32,62*) and found that an exposure concentration of 800 µg/m^3 gave a reasonable fit to the measured benzene breath concentrations, as shown for smoker 1 (1st cigarette) in Figure 7.

The modeled plot in Figure 7 was developed by first obtaining the τ_i and a_i parameters of the model from the decay portion of the curve, then using these values to estimate the cyclic uptake and decay segments (*55*). The discrete puffs on the cigarette during the exposure period are responsible for the sawtooth appearance of the plot. The cycles are sufficiently brief that the breath concentrations preceding the 2nd, 3rd, 4th, and 5th puffs are nonzero and are the reason that

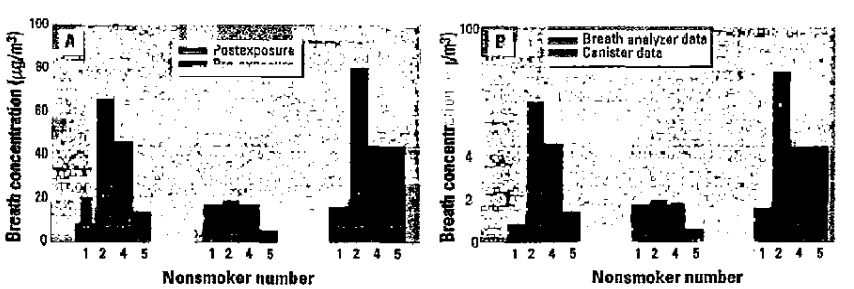

Figure 6. Effect of exposure to ETS on concentrations of target compounds in exhaled breath of nonsmokers: (*A*) Pre- and postexposure breath levels measured using the real-time breath analyzer. (*B*) Comparison of postexposure breath levels obtained using the real-time breath analyzer with whole-breath samples collected directly in canisters and analyzed by GC/MS.

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both the measured and modeled plots show a distinct buildup in breath level with each puff at the upper as well as at the lower end of each cycle. After the 5th and final puff, the exposure-free "washout" period is sufficiently long that the breath concentration returns to the preexposure baseline level.

If we now assume that a typical smoker smokes a cigarette in 10 min every 30 min throughout a 16-hr day (equivalent to a 1.5 pack/day smoker), followed by an 8-hr overnight exposure-free period, we can estimate the concentration of a tobacco smoke constituent in the exhaled breath of the smoker from long-term cyclic exposure to the chemical. For these calculations, we assumed a four-compartment model with τ_1–τ_4 = 3, 30, 180, 3,600 min; a_1–a_4 = 0.25, 0.25, 0.25, 0.25; f = 0.2; and C_{air} = 500 μg/m^3. The results are summarized in Figure 8, which shows the smoker's breath levels immediately before and after each of the 32 cigarettes smoked during the day, for days 1 and 2 and days 14 and 15. In keeping with the results obtained experimentally, the breath concentration increases very rapidly with the first few cigarettes, drops down to a level between 2 and 10 μg/m^3 during each 8-hr exposure-free period, and slowly rises to reach a constant maximum value (at ~50 μg/m^3) on day 15. For this scenario, the long-term values just before the last cigarette of the day, presented in Figure 8, are similar to the values observed earlier with smokers in the TEAM studies, for whom average benzene breath concentrations, for example, were 14 μg/m^3 (*18*).

Nonsmokers. Although most of the preexposure measurements made on the nonsmokers with the real-time breath analyzer were below the limits of quantitation, as were some of the postexposure measurements, the data in Figure 6A show that in all cases, except one, the postexposure concentrations were higher than the preexposure levels. It is not clear why there was no increase in the breath benzene concentration after exposure in the case of nonsmoker 1. The preexposure breath of nonsmoker 2 contained measurable amounts of benzene and 2,5-dimethylfuran. Because 2,5-dimethylfuran is not normally measurable in the breath or blood of nonexposed nonsmokers (*38,39*), its presence in the breath of this subject before these measurements were made suggests that he may have misrepresented himself as a nonsmoker.

We also collected postexposure breath samples in canisters and analyzed them by GC/MS, usually within a few days of collection, for comparison with the breath analyzer data. The results are shown in Figure 6B. The canister samples confirmed the presence of the target compounds in the postexposure breath of the nonsmokers. These breath samples were collected directly from the subject and did not pass through the filter or tubing of the breath analyzer.

In virtually every case except two, the canister concentrations were lower than the levels measured with the breath analyzer. Although the canister samples were collected immediately after the real-time measurements, it is unlikely that the differences between the two measurements were caused by changes in concentration with time because the postexposure canister collections took place over a relatively flat segment of the decay curve (Figures 1 and 4). As a result, changes in breath levels with time after exposure are assumed to have been insignificant.

For benzene and 1,3-butadiene, the difference between the canister and the continuous analyzer may be caused by contamination of the quartz fiber filter and inner walls of the analyzer tubing with condensed tar from cigarette smoke that occurred immediately before these measurements were made, during the smoker's exposure session. This condensate, which presumably adsorbs the target gases during the exposure periods, desorbs some of these organic vapors when uncontaminated air or the nonsmoker's breath is then passed through the filter. Measurements of pure air flowing through the analyzer, both with and without a clean filter, showed that the presence of the filter did not contribute to the background levels. Furthermore, passing a standard of the target analytes in air through the filter and tubing, followed by humidified pure air, showed that the filter did not retain these VOCs. Because of time constraints, we were unable to replace the filter and clean the analyzer system before switching from the smoker to monitor the nonsmoker's breath. It is reasonable to conclude, therefore, that for these two compounds the higher breath analyzer levels observed in Figure 6B may have derived from tar buildup during each exposure session.

This explanation is not sufficient for the much greater difference the two methods display for 2,5-dimethylfuran. Using the fragment ions at *m/z* 95 and 67, we were able to detect 2,5-dimethylfuran unambiguously in our gas standard using the real-time tandem MS/MS technique. However, tobacco smoke is an extremely complex mixture, and recent GC/MS analyses of smokers' breath conducted in our laboratory have shown that tobacco smoke also includes several other compounds that contain *m/z* 95 and 67 as fragment ions, such as camphene

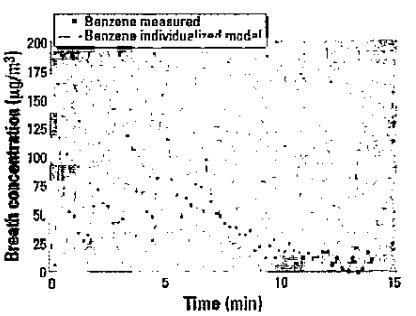

Figure 7. Measured and modeled uptake and decay of benzene in breath of smoker 1 while smoking first cigarette. See "Discussion" for details.

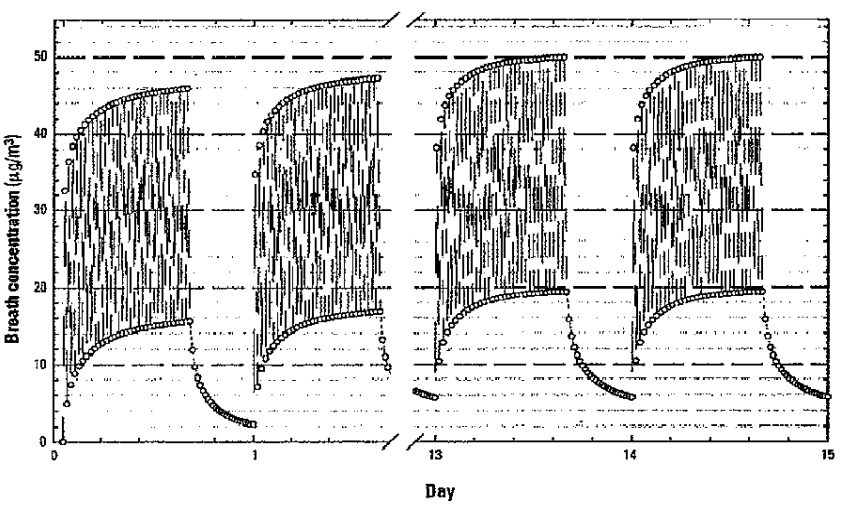

Figure 8. Modeled concentration of tobacco smoke constituent in exhaled breath of a regular smoker due to cyclic exposure as a result of smoking 32 cigarettes in a 16-hr day (i.e., one cigarette in 10 min every 30 min throughout the day, followed by an 8-hr overnight exposure-free period).

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(1,7,7-trimethylbicyclo[2.2.1]heptane), limonene [1-methyl-4-(1-methylethenyl)-cyclohexene], and menthol [5-methyl-2-(1-methylethyl)-cyclohexanol] in the case of mentholated cigarettes. Because the MS/MS technique is unable to distinguish among different chemical species with identical parent/daughter transitions, the masses we used here measured the sum of the contributions from 2,5-dimethylfuran, camphane, limonene, and others. Although we chose *m/z* 95 and 67 at the time to give maximum sensitivity for 2,5-dimethylfuran, subsequent experiments have shown that monitoring the *m/z* 96/67 parent/daughter transition would have yielded greater specificity and adequate sensitivity for these analyses. Further tests indicate that all of the compounds that contribute to the ion signals at m/z 95 and 67 occur only in tobacco smoke, i.e., in active or passive smokers' breath, and are not detected at all in the breath of unexposed smokers. It is reasonable to assume, therefore, that the much greater difference observed for 2,5-dimethylfuran between the two measurement methods is due largely to constituents present in the smoke that contributed as interferents under the monitoring conditions we used in this work. However, the generally good agreement obtained for 1,3-butadiene between the two techniques indicates that further validation of these methods for 2,5-dimethylfuran in the tobacco smoke breath matrix is needed.

As noted earlier, the response of the ion trap was set to accommodate the high concentrations of the compounds in the smokers' breath, and its sensitivity was not optimal for measuring the lower concentrations of the target compounds in the breath of the nonsmokers. To monitor these much lower levels effectively would have required the development and implementation of a strategy for optimizing the ion trap operating parameters (ionization or "gate open" time, excitation time, excitation voltage, low-mass cut-off value, ion source temperature, mass range, and the like) (*51,52*). For the present application, which required us to switch rapidly from the smoker to the nonsmoker during the exposure sequence, it was impractical to make these careful adjustments reproducibly to the instrument parameters, so the same conditions were used for each smoker/nonsmoker pair. It was largely for this reason, rather than the result of any inherent limitation in the sensitivity of the ion trap mass spectrometer, that most of the nonsmoker preexposure measurements made with the breath analyzer were below the limits of quantitation.

Although the breath analyzer may have overestimated the postexposure breath concentrations of the nonsmokers, for the reasons noted above, we nevertheless used the data to compare the background-corrected concentrations of benzene, 1,3-butadiene, and 2,5-dimethylfuran in the breath of the nonsmoker and smoker during each 2- to 2.5-hr smoking session. The average increases, along with their standard deviations, are summarized in Table 3. For each compound, except 2,5-dimethylfuran for the nonsmokers, the mean value is greater than two standard errors of the sample mean, indicating with about 95% confidence that the exposure increases were significantly different from zero. The results also show that for our experimental conditions, the increase in the target compound concentrations in the smokers' breath after four cigarettes was at least 12–24 times that in the nonsmokers' breath.

Conclusions

This study includes what are probably the first measurements of the carcinogen 1,3-butadiene in human exhaled breath. The average peak concentrations of 1,3-butadiene and benzene in the smokers' breath were 360 and 522 μg/m^3, respectively. The peak values obtained in this study for breath benzene are considerably higher than those previously reported in the literature. The two-compartment model fits both the decay data and the maxima after each cigarette fairly well for all three target chemicals. The model furthermore predicts that the peak values will be reached after about 12–15 cigarettes have been smoked and that the values will return to baseline after the 8-hr sleep period.

All three target compounds were detected in the breath of the nonsmokers after exposure, using two different types of sample collection and analysis techniques. However, these measurements indicated that there was some contamination of the breath analyzer, probably due to the buildup of condensed tar in the system. Future studies of nonsmokers based on the breath analyzer will need to take special precautions to avoid this effect.

Table 3. Average increase in breath concentrations of benzene, 1,3-butadiene, and 2,5-dimethylfuran (μg/m^3)[a] experienced by smokers and nonsmokers during smoking sessions.

Compound	Nonsmokers ($n = 4$)[b]	Smokers ($n = 5$)
Benzene	25.7 ± 12.9[c]	478 ± 144
1,3-Butadiene	14.4 ± 5.3[d]	353 ± 24
2,5-Dimethylfuran	27.1 ± 18.1[e]	340 ± 71

[a]Mean ± SD. [b]$n = 3$ for benzene. [c]Includes one preexposure and one postexposure measurement below limit of quantitation (LOQ). [d]Includes two preexposure measurements below limit of detection (LOD), and two pre- and one postexposure measurement below LOQ. One-half the detection limit was used for measurements below the LOD. [e]Includes two pre- and one postexposure measurement below LOQ.

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